7

Label-free Biosensors for Health Applications

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1. Introduction

Biosensors are widely used for health applications. Indeed, the current success of biosensors is attributed to the extraordinary demands of disease diagnoses and control, as well as the ability of biosensors to offer a convenient, hygienic, rapid, and compact method for personal monitoring. Biosensors offer enormous potential for detecting a wide range of analytes in the health care and food industries and in environmental monitoring. As quoted, MicroChips president John Santini expects the technology to be used as in-the-flesh physicians within 10 years: "It's a very exciting time," he says. "Our next step is a manually, wirelessly controlled biosensor that detects and treats an acute condition, and then a biosensor that will approximate an artificial organ; it'll sense a condition and respond automatically without user intervention." In this chapter, we review applications and advances in biosensor technology, focusing on four applications in the health field: 1) investigation of the interaction of antigens with antibodies produced in healthy and diseased subjects, 2) disease markers and virus detection, 3) clinical diagnosis and control of emerging infectious diseases, and 4) market potentials. Specifically, we discuss the application of a label-free biosensor based on ellipsometry in the development of future biosensors, the current and future clinical applications of this technology, and its viability. The goal of this chapter is to provide a brief description of the role of biosensors in *in vitro* diagnostics and scientific research related to the health field. Readers interested in competing or related technologies (e.g., ellipsometry, microfluidics, and surface modification technologies) are referred to one of several excellent recent reviews (Jin et al., 2011; Qi et al., 2009a; Zhang, et al., 2005). In the following section, health applications are described using a label-free biosensor based on ellipsometry.

2. Label-free biosensor based on ellipsometry

2.1 Biomedical application history

The first publication concerning biological application of antigen and antibody is a paper by Langmuir and Schaefer dating back to 1936 (Hans, 1998, as cited in Langmuir& Schaefer, 1936). The interaction between an antigen-immobilized substrate and the corresponding

antibodies was observed with an optical technique. Although this original 'immunosensor' was over 59 years old at the time, the concept of a biosensor was first brought forward in 1995 by Jin and Hans and used to study biomolecular interactions (Jin et al., 1995, 1996). A biomolecule layer composed of a common protein, such as fibrinogen, human serum albumin, or human immunoglobulin G, was spread on the surface of the sensor. The interaction between general proteins such as fibrinogen and an antibody against fibrinogen was then investigated. However, biosensor detection of clinical samples was only recently developed, such as detection of disease markers and viruses and investigation of interactions between antibodies and antigens related to clinical diagnoses (Jin et al., 2011; Qi et al., 2009a).

2.2 Technical characteristics of the biosensor based on ellipsometry

The principle of the biosensor has been discussed in several reports (Jin et al., 2011; Z.H. Wang et al., 2006). Here, we list some key technical characteristics of biosensors based on ellipsometry:

- Label-free

When the interaction between biomolecules occurs, the variation of the molecular mass surface concentration on the surface is identified by the biosensor based on ellipsometry without label (e.g., the horseradish peroxidase used in enzyme-linked immunosorbent assays).

- High-throughput

Combined with a microfluidic array reactor, which fabricates the chips, the biosensor based on ellipsometry has become an automatic and high-throughput system by adding ligand, washing, blocking, and reacting samples. Recently, an 8×6 biomolecule reactor-array was developed as a promising technique for a parallel protein assay. The 48 protein arrays in the 8×6 matrix are shown in Figure 1 (Jin, 2008). Interaction of common protein, detection of five hepatitis B virus markers in patient serum, detection of different ladder concentrations, and the detection sensitivity of CD146 (known as the melanoma cell adhesion molecule or cell surface glycoprotein MUC18) (Guezguez B, 2007) are presented on the chip.

- Rapid

Using the automatic program of the microfluidic array reactor to add ligands, washing, blocking, and reacting samples, ligands screening and detection of markers can be accomplished in 1 to 2 h.

- Low sample consumption

Consumption of ligands and samples is on the microliter level. For example, in hepatitis B virus detection, hepatitis B virus ligand consumption is 10 μl/area (The area is a small squareness area, see Figure 1.), and hepatitis B virus serum consumption is 40 μl/area (Qi, et al. 2009a). Enzyme-linked immunosorbent assays in milliliter level require a larger volume of the same concentration.

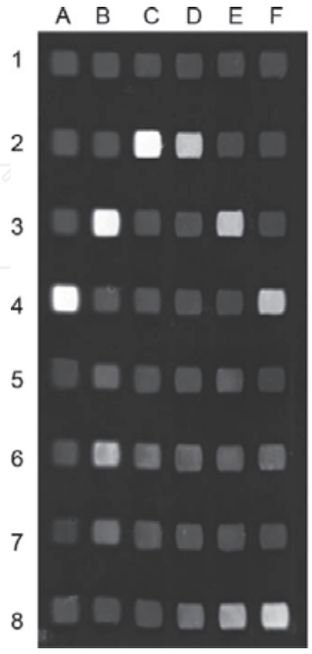
- Low damage to the biomolecules

The biosensor works via an optical, reflection-based technique that uses polarized light to determine the optical properties of a sample (Z.H. Wang, et al., 2003a). It is almost "touch-free" to read the detection result, so there is a decreased effect to bioactivity than, for instance, atomic force microscopy and surface-enhanced laser desorption/ionization.

- High sensitivity

The biosensor displays different detection sensitivity toward different samples. For example, the sensitivity of the biosensor for detecting antigen markers, such as hepatitis B

virus surface antigen, reaches 1 ng/ml (Qi, et al. 2009a), while the detection sensitivity of CD146 is < 1 ng/ml (see Figure 1: F5, F6, and F7 areas).



Array	Detailed reactants
C1	Ligand HBsAb
B1	HBsAg negative sample / Ligand HBsAb
A1	HBsAg standard sample (1ng/ml) / Ligand HBsAb
F1	Ligand HBsAg
E1	HBsAb negative sample / Ligand HBsAg
D1	HBsAb standard sample (1ng/ml) / Ligand HBsAg
A5, B5, C5, D5, E5	Ligand five marks of hepatitis (HBcAg,HBeAg,HBeAb,HBsAg, HBsAb)
A6, B6, C6, D6, E6	Hepatitis positive serum / Ligand five marks of hepatitis
A7, B7, C7, D7, E7	Hepatitis negative serum / Ligand five marks of hepatitis
A8, E2, F2, F3, D3, D4, E4	Ligand IgG
D2, E3, F4	Anti-IgG / Ligand IgG
B8-F8	Anti-IgG (with concentration of 1, 10, 10^3, 10^4, 10^5ng/ml) / Ligand IgG
A2, A3, B2, B4, C3, C4	Ligand FIB
C2, B3, A4	Anti-FIB / Ligand FIB
F5	Ligand CD146 (AA98)
F6,F7	CD146 positive serum (< 1ng/ml) / Ligand CD146

Fig. 1. Forty-eight protein arrays in a matrix. Left: The visual result of a protein micro-array. Right: The detailed reactants relative to the left graph (Jin, 2008).

- Automatic control

Some parameters of the microfluidic array reactor, such as the position number of the sample plate, flow velocity in the microfluidic array reactor of sample or ligand, time of immobilization or reaction, and number of cycles, can be edited in the automatic program.

- Visualization of results

Visualized gray-scale images are offered by imaging ellipsometry in several seconds, which is shown on a computer screen. The target interacting ligands on the surface can be identified by values in gray-scale with associated software.

- Quantitative detection

Combined with the calibration curve method, disease markers and viruses in samples can be detected easily and quantitatively with the label-free biosensor.

2.3 Operational process of the biosensor based on ellipsometry

The operational process of the biosensor, which includes surface modification, ligand immobilization, biomolecule interaction, and result reading, is shown in Figure 2.

Surface modification is a process by which chemical reagents for reactive groups on the silicone surface for biomolecule immobilization Surface modification has two obvious functions: one is the presentation of ligand on the biosensing surface; and the other is to prevent nonspecific binding (Jin, 2008). Presently, surface modification methods include physical adsorption, chemical covalent immobilization, and biologic modification. Physical adsorption is seldom used because the immobilized proteins suffer partial denaturation and tend to leach or wash off of the surface and compete with adsorption (Bhatia, et al., 1989).

Chemical covalent immobilization is often used to immobilize proteins due to the strong, stable linkage, and biological modification is a future direction because it provides oriented immobilization and better biological compatibility. Aldehyde modification, carboxyl modification, and biologically oriented immobilization are often used in biomedical applications (Qi, et al., 2010, 2009b; Z.H. Wang & Jin, 2004, 2003a).

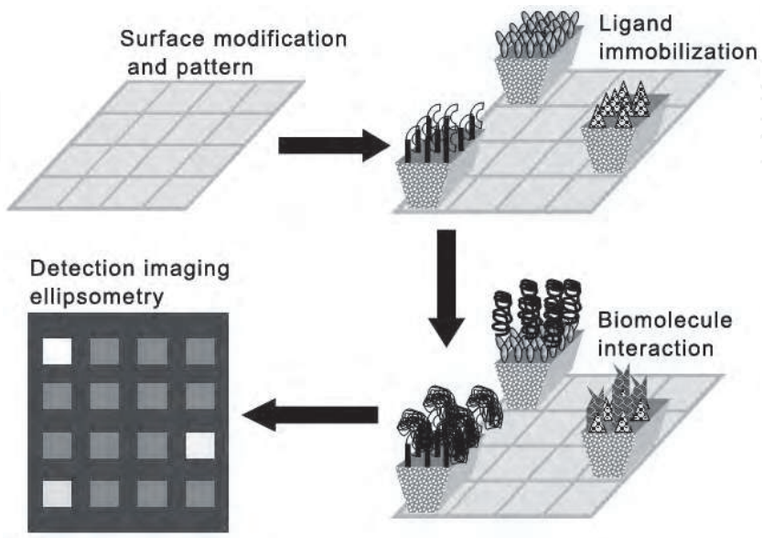


Fig. 2. Operational process of the biosensor based on ellipsometry. Antibodies (or antigens) can be immobilized as ligands to each patterned area as a bio-probe on the modified surface of a silicon substrate. Each bio-probe can capture its corresponding antigens (or antibodies) in a test sample pumped by microfluidic reactor. When the corresponding antigens (or antibodies) in the solution interact with the bio-probes, forming a complex, the surface concentration becomes higher than the initial bio-probe layer. The distribution of the lateral protein layer pattern is simultaneously detected by imaging ellipsometry, which may further point to the existence of antigens (or antibodies) in the tested solution (Jin, 2008).

3. Applications in the health field

In the field of human health, there is an increasing demand for inexpensive and reliable sensors to quickly detect and analyze various and rapidly changing disease markers. For example, patients frequently display rapid variations in biochemical levels of disease markers such as C-reactive protein that require instant assays to detect. Indeed, early detection and diagnosis can be used to greatly reduce the cost of patient care associated with the advanced stages of many diseases. More than a hundred types of proteins recognized as diseases markers can be detected by traditional analytical techniques such as enzyme-linked immunosorbent assays. However, based on the above features of the ellipsometry-based biosensor, it has also been widely used to detect and monitor biomolecule interactions, especially for biomedical applications. A sample focusing on tumor marker detection is shown in Figure 3.

The ability to detect pathogenic and physiologically relevant biomolecules in the body with high sensitivity and specificity offers the opportunity for early diagnosis and treatment of diseases.

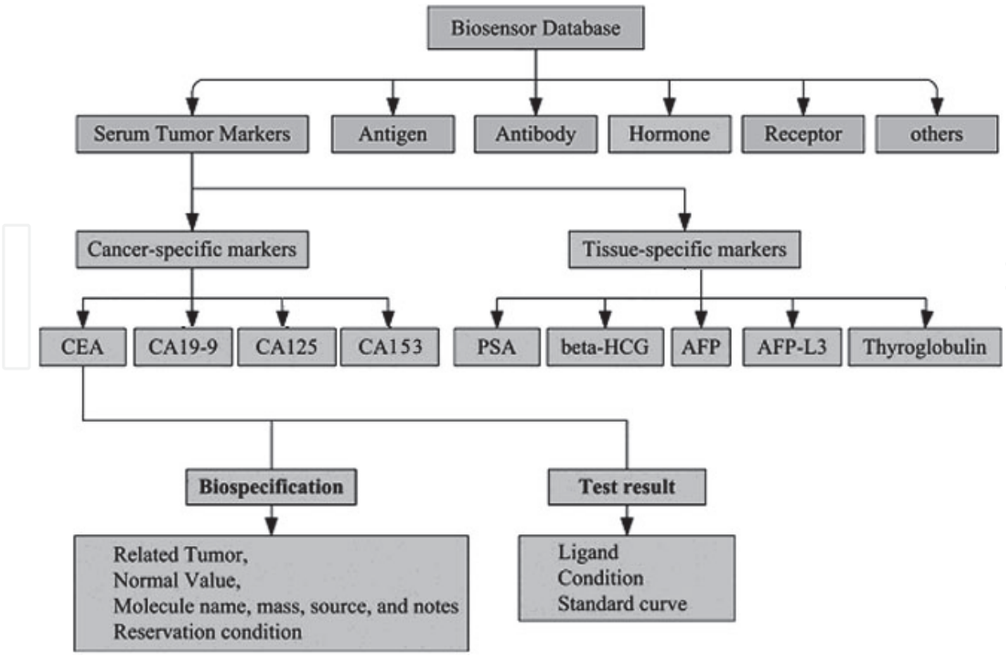


Fig. 3. Application of the biosensor. The use of biosensors to detect tumor markers in serum has spread widely (Jin, 2011).

3.1 The interaction of antigens with antibodies in healthy and diseased subjects

The initial impetus for advancing biosensors based on ellipsometry came from detection of the interaction of general antibodies and antigens, and some basic methods have been established, such as the ligand immobilization, high specificity probe screening, protein delivery, biomolecule affinity presentation on a chip, specific interactions, the influence of nonspecific binding, detection sensitivity, sample consumption, and calibration curves for quantitative detection.

3.1.1 Detection of antigen-antibody interactions

In biomedicine, human fibrinogen, hepatitis B surface antigen, human immuoglobulin G, and human serum albumin are often used as mode proteins. Using the aforementioned proteins as models with the biosensor, the feasibility is shown in Figure 4. Significant increases of gray-scale value appear in the square areas exposed to the corresponding target (Jin, et al., 2003). These results demonstrate that target samples can be identified by the ellipsometry-based biosensor.

3.1.2 Real-time detection of the antibody-antigen interaction

The biosensor based on ellipsometry can monitor protein interactions in situ and in real time to provide protein interaction kinetics information, such as association rate, dissociation rate, and affinity constants. Some special operation details of real-time detection are shown.

- Model proteins were prepared and immobilized on the substrate;
- The chip was inserted into the reaction cell;
- A mixture of antiserum containing corresponding antibody was poured into the reaction cell;

- A series of images (in gray-scale) of several binding processes between antibodies in solution and antigens were recorded by the biosensor; and
- The surface concentrations of analytes in the analytical areas of each image were measured and plotted versus time to determine the real-time binding curves.

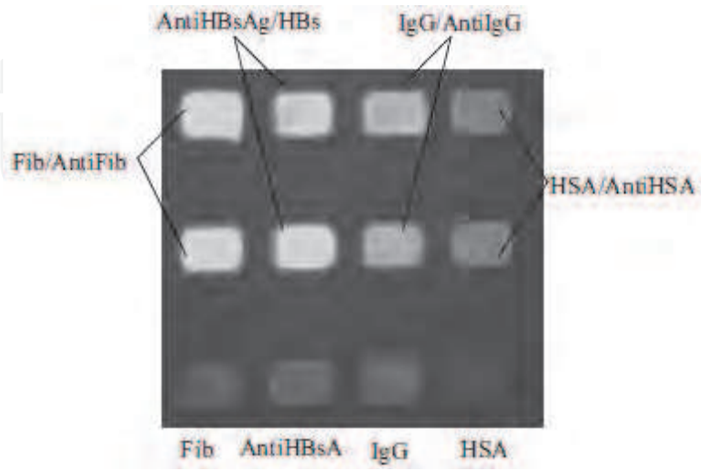


Fig. 4. Detection of several model proteins using the biosensor based on ellipsometry. Model proteins Fib, AntiHBsA, IgG and HSA were immobilized in four different columns, respectively. Phosphate-buffered saline was added to one area as a reference control. Corresponding target was then added to the other two areas in the column. (Z.H. Wang & Jin, 2003b)

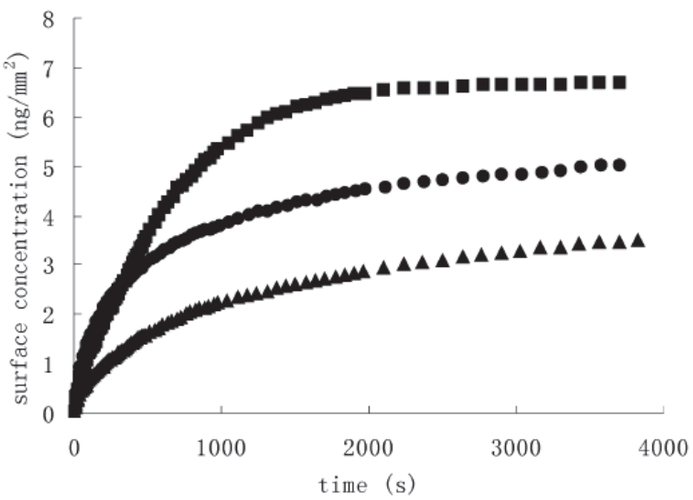


Fig. 5. Binding curves of anti-fibrinogen/fibrinogen (■), anti-human immunoglobulin/human immunoglobulin (●), and anti-human serum albumin/human serum albumin (▲) obtained by the biosensor (Z.H. Wang & Jin, 2003b).

The real-time binding curves are shown in Figure 5. Detailed data processing and kinetics analysis was performed according to the method described in the literature (Malmborg, et al., 1992). In a clinical setting, a patient's serum is a mixture similar to that used to generate

Figure 5, containing antibodies against fibrinogen, human serum albumin, and human immunoglobulin. A chip contains many immobilized ligands that bind to the same marker in serum but with different binding affinities. The biosensor offers a convenient way to compare these ligands' binding affinities under the same conditions, and ligands with high affinity can be screened. The convenient way comparing these ligands' binding affinities might compare the effectiveness of drug and screen drug, so the ability to sense multiple interactions in real-time makes the biosensor particularly well suited for monitoring disease progress, screening for highly effective drugs, and understanding disease mechanisms.
The interaction of antigens and antibodies produced in healthy and diseased subjects (e.g., hepatitis B markers antibodies and antigens (Qi, et al., 2009a), severe acute respiratory syndrome virus particles and antibodies (Qi, et al., 2006a), ricin antibody screening (ricin found in castor beans is one of the most potent plant toxins) (Niu, et al., 2010), and others) has been studied by the biosensor based on ellipsometry. These studies demonstrate the biosensor's use for health applications.

3.2 Disease markers and virus detection

Protein markers should be specific and sensitive and have prognostic value. Efforts to discover disease markers have focused on elucidating serum molecules that have diagnostic and prognostic value (Schena, 2005). High-throughput biosensors, including the biosensor based on ellipsometry, may shorten the time required to find disease markers. In this respect, biosensors are the best choice among the current techniques.

3.2.1 Qualitative detection of five hepatitis B virus markers

Hepatitis B virus is a human hepadnavirus that causes acute and chronic hepatitis and hepatocellular carcinoma (Bai, et al., 2003). The detection of hepatitis B virus markers is clinically important for the diagnosis of infection with this virus (Chen, et al., 2006). Five markers of hepatitis B virus (including hepatitis B surface antigen, the hepatitis B surface antibody, hepatitis B e antigen, hepatitis B e antibody, and hepatitis B core antibody) are a group of general markers used in the monitoring of hepatitis B virus infection. Following key steps of detection markers were operated for clinical application:

- Screening for highly effective probes;
- Detection sensitivity; and
- Optimization of detection conditions.

Presently, several probes can be simultaneously compared by the biosensor on one chip, which is shown in Figure 6. For the same target, different probes present different values in the grayscale, which indicates that the various probes have different bioactivities. Thus, highly effective probes were found for sensitive clinical diagnosis.
Sensitivity is important for hepatitis B marker detection. Hepatitis B surface antibody and hepatitis B surface antigen national positive reference samples (from the National Institute for the Control of Pharmaceutical and Biological Products (China)) were detected by the biosensor in 2009. The detection sensitivity of hepatitis B surface antigen is 1 ng/ml, and the detection sensitivity of hepatitis B surface antibody is > 1 IU/ml. Thus, the sensitivity has already reached clinical standards.
The biosensor based on ellipsometry permits multiplexed analysis. It can detect the five Hepatitis B markers of several patients simultaneously in about 1 h, proving its feasibility in clinical diagnosis. High affective probes increase sensitivity and resolving power. Other

biosensor advantages, such as higher sensitivity, a simplified process, and short test time, are also significant for rapid diagnosis.

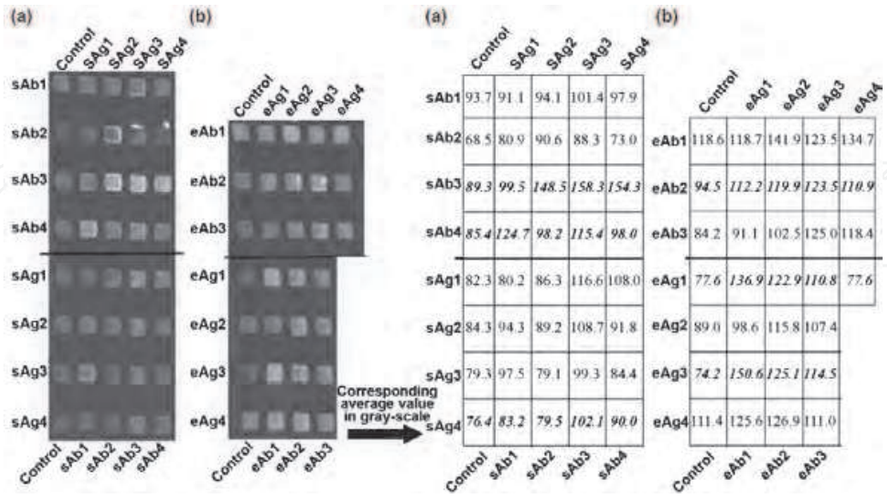


Fig. 6. Screening of hepatitis B ligands. (a) Screening of hepatitis B surface antibody and hepatitis B surface antigen. Different lots of hepatitis B surface antibody (sAb) and antigen (sAg) ligands were first immobilized in different rows. After blocking with BSA, the first row was used as a control. Different lots of hepatitis B surface antigen and hepatitis B surface antibody markers were detected in different rows. (b) Screening for hepatitis B e antibody and hepatitis B e antigen. The *italics* indicate results with the largest variation in gray-scale values, which in turn indicate that the ligands had higher bioactivity (Qi, et al., 2009a).

3.2.2 Quantitative detection of breast cancer marker: Carbohydrate antigen 15-3

In 2008, an estimated 636,000 cases of breast cancer were diagnosed in high resource countries, while an additional 514,000 cases were diagnosed in low and middle resource countries, where it has now become the most common female cancer (El Saghir, et al., 2011). Carbohydrate antigen 15-3 is frequently measured as a breast cancer marker test using the biosensor based on ellipsometry (Zhang, et al., 2005). According to Figure 2, quantitative analysis of carbohydrate antigen 15-3 was performed using the calibration curve method:

- A serum sample with a known concentration of carbohydrate antigen 15-3 was serially diluted;
- These various concentrations were detected;
- A calibration curve was drawn using the 15-3 concentration as the abscissa and the gray-scale value as the vertical axis;
- An unknown sample was analyzed; and
- The concentration of 15-3 in the unknown sample was determined with the calibration curve.

The concentration of carbohydrate antigen 15-3 in a serum sample had been determined by an electrochemiluminescence immunoassay. The serum sample with known concentration is used as standard sample to make a calibration curve of the biosensor. The calibration curve of carbohydrate antigen 15-3 detection is shown in Figure 7. The index period of the curve is 0~20 kIU/L, corresponding to gray-scale values of 58~99. If the concentration exceed the

detection scope (0~20 kIU/L), the unknown test samples must be diluted; the lower limit of detection is 1 kIU/L. The realization of quantitative label-free detection of a cancer marker may aid in earlier diagnosis, monitoring the course of the disease, even exploring the mechanism of cancer.

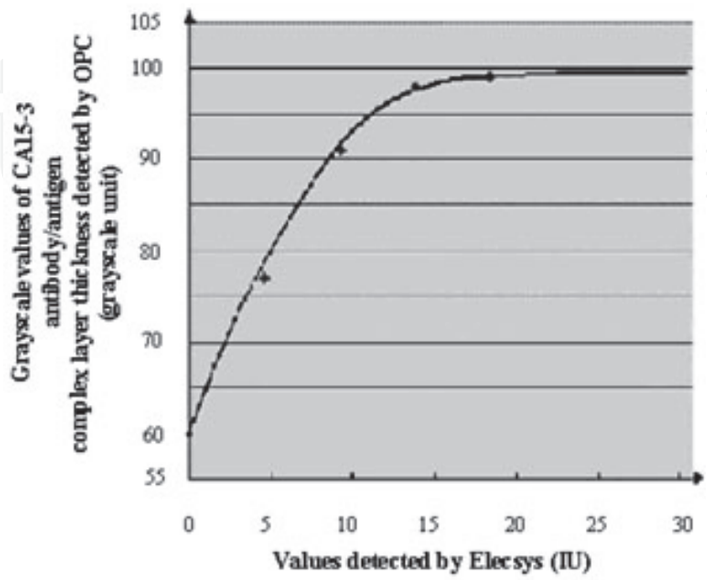


Fig. 7. Calibration curve for carbohydrate antigen 15-3 concentration detection (Zhang, et al., 2005).

3.2.3 Detection of tumor markers

Analyzing only one tumor marker is insufficient to diagnose cancer in 2010, a review exhibited a novel co-detection of three common tumor markers: alpha-fetoprotein, alpha-L-fucosidase, and ferritin (Jin, 2011). Thus, quantitative analysis was performed by the biosensor with the following calibration curve method:

- A chip was designed to simultaneously detect three markers in a sample;
- A calibration curve for the biosensor was plotted;
- A cut off value was determined by the receiver operating characteristic; and
- The three markers in a clinical serum sample were examined on a chip.

Detection results of several patients' markers were compared and analyzed. Sensitivity reached the ng/ml or U/L level. Thirty-two normal sera and 24 liver cancer patient sera were quantitatively analyzed. The realization of simultaneous detection of several markers by the biosensor may increase diagnostic specificity in a clinical setting.

3.2.4 Detection of phage M13KO7 for building virus a detection model

Phages are estimated to be the most widely distributed and diverse entities in all reservoirs populated by bacterial hosts. In 2009, Phage M13KO7 was detected by the biosensor based on ellipsometry as a model for virus detection. A highly versatile and powerful virus detection platform has been established (Qi, et al., 2009b). Based on common antibody/antigen or disease marker detection, three key steps (e.g., ligand immobilization, sensitivity detection, and microscopic confirmation) were optimized.

The avidin/biotin method (Fig. 8) was chosen to immobilize the antibody bio-GP3 against phage M13KO7. The avidin/biotin immobilization method is often used in other immunoassays (Vijayendran & Leckband, 2001). It has several advantages: 1) ligands are strongly immobilized because biotin and avidin can specifically interact with stronger affinity (~$10^{15}M^{-1}$) than the antibody-antigen interaction (~10^{5}-$10^{12}M^{-1}$) (Friguet, et al., 1985; Malmborg, et al., 1992); 2) immobilization is oriented, which helps antibody display its Fab domain for improved sensitivity; and 3) it may offer a more physiological environment.

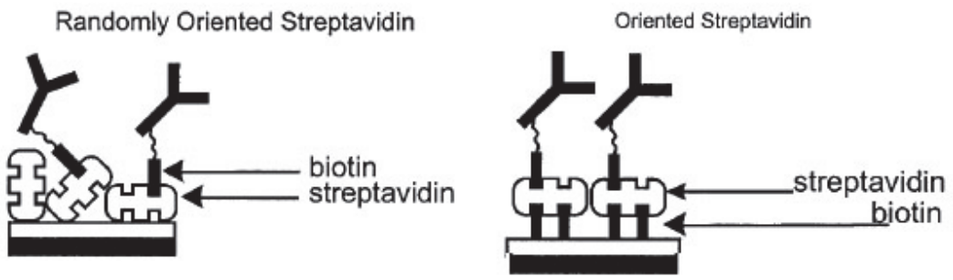


Fig. 8. Avidin/biotin immobilization method (Vijayendran & Leckband, 2001).

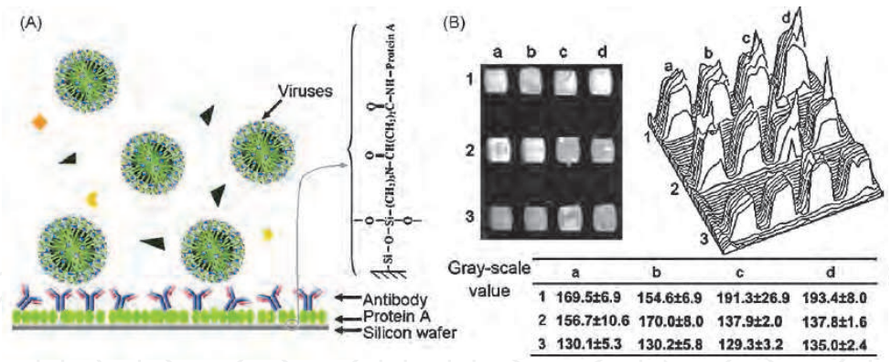
The sensitivity of phage M13KO7 detection can reach 10^{9} plaque forming units/ml. Phage detection results by the biosensor have been confirmed with atomic force microscope. Imaging indicates that the biosensor can capture whole viruses, not just fragments. Thus, the virus detection biosensor platform has potential applications for human health.

3.2.5 Detection of avian influenza virus

According to World Health Organization statistics, the number of cases of avian influenza virus H5N1 directly crossing barriers and infecting humans was 534, causing 316 deaths by March 2011 (World Health Organization, 2011). Avian influenza virus subtype H5 can be detected with the biosensor based on ellipsometry using the above virus detection platform.
The oriented immobilization of probe was realized using protein A and antibody for avian influenza virus detection. Figure 9 (A) shows the probe immobilization method. This is a kind of biological immobilization, which also offers a more physiologically relevant environment to maintain the bioactivity of the probe (Qi, et al., 2010). The results show that 4A4 antibody can react specifically with avian influenza virus subtype H5N1, while CAM4 can interact with both H5N1 and H9N2.
The sensitivity of H5N1 detection is 2.56×10^{-3} tissue culture infectious dose/ml, which is more sensitive than a lateral-flow immunoassay (Remel Inc.). The corresponding areas were scanned with near-field optical microscopy. The microscopic evidence is presented in Figure 10, showing that intact avian influenza virus particles were bound. Direct virus detection may help with earlier diagnosis than disease marker detection.

3.2.6 Detection of other disease markers and viruses

C-reactive protein (Zhu, et al., 2007), soluble angiopoietin receptor Tie-2 (C.L. Wang, et al., 2009), thymidine phosphorylase (Li, et al., 2004), Alzheimer's disease tau protein (Qi, et al., 2006b), and others had also been detected using the biosensor. These diseases markers are closely related to human health. Thus, qualitative or quantitative detection with the biosensor can aid in earlier disease diagnosis and improve health.



Gray-scale value	a	b	c	d
1	169.5±6.9	154.6±6.9	191.3±26.9	193.4±8.0
2	156.7±10.6	170.0±8.0	137.9±2.0	137.8±1.6
3	130.1±5.3	130.2±5.8	129.3±3.2	135.0±2.4

Fig. 9. Detection of avian influenza virus samples using the biosensor based on ellipsometry. (A) Schematic illustration. Avian influenza virus antibody is immobilized on the substrate. (B) Experimental image in gray-scale and a 3-D gray-scale distribution map. Antibody CAM4 was immobilized in columns 'a' and 'b'; 4A4 in columns 'c' and 'd'; H5N1, H9N2 and the control are shown in rows '1', '2', and '3', respectively (Qi, et al., 2010).

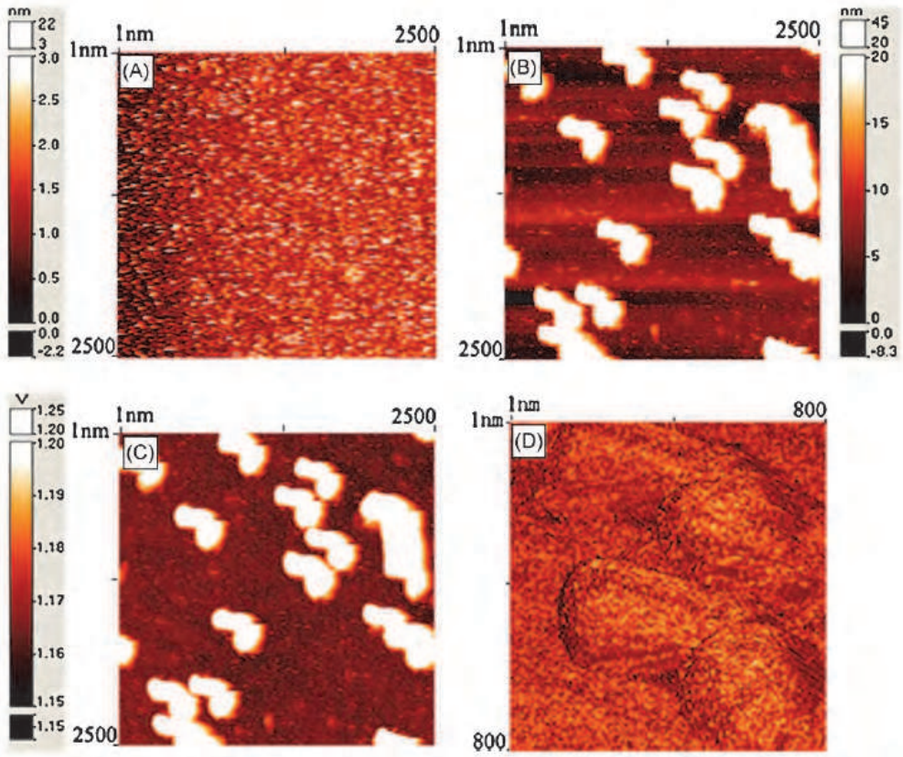


Fig. 10. Near-field optical microscopy images of H5N1. (A) and (B) Shear force mode images for H9N2 and H5N1, respectively. (C) Reflection mode image of H5N1. (D) 3-D reflection mode image of H5N1 (Qi, et al., 2010).

3.3 Clinical diagnosis and control of emerging infectious diseases

The ability of the biosensor based on ellipsometry to detect antibodies or antigens, disease markers, and viruses from patient samples with high sensitivity and specificity offers a

powerful opportunity in early diagnosis and treatment of diseases. Related clinical applications have begun.

3.3.1 Clinical diagnosis of hepatitis B patients' sera

Hepatitis B virus infection is the most common cause of chronic liver diseases; an estimated 350 million people are chronically infected with hepatitis B virus worldwide (Sun, et al., 2002). Further, hepatitis B virus infection plays an important role in the development of hepatocellular carcinoma (De Mitri, et al., 2010). A rapid, simple, and direct method is urgently needed for clinical hepatitis B diagnosis. In section 3.2.1, the screening probe, standard national reference sample detection, and highly sensitive hepatitis B detection results demonstrated that the biosensor based on ellipsometry is feasible for clinical diagnosis of the disease (Z.H. Wang, et al., 2006; Jin, et al., 2004). Thus, the application of the biosensor based on ellipsometry could greatly enhance hepatitis B detection speed.

Cut-off values are important for clinical diagnosis of hepatitis B and it detection by the biosensor based on ellipsometry. The cut-off value can help us to distinguish between strong positive, near cut-off, and negative samples. Other diagnosis techniques, such as enzyme-linked immunosorbent assays, have cut-off value instructions included in the assay kits (Qi, et al., 2009a). The cut-off value of the biosensor was determined with a receiver operating characteristic curve. With the cut-off value, the detection of five hepatitis B virus markers by the biosensor was consistent with enzyme-linked immunosorbent assays.

Sera from 169 patients were analyzed with the biosensor for the purpose of clinical diagnosis. Samples from 60 patients included clinical information of hepatitis B from Shandong Provincial Hospital from qualitative enzyme-linked immunosorbent assay detection results (the assay kit was produced by Shanghai Rongsheng Biotech Co. Ltd). The remaining samples were from patients from the Tientsin Blood Disease Hospital and also included clinical information of hepatitis B (the assay kit was produced by Beijing Wantai Co Ltd.) Figure 11 shows the detection results of 109 hepatitis B patients' sera samples from

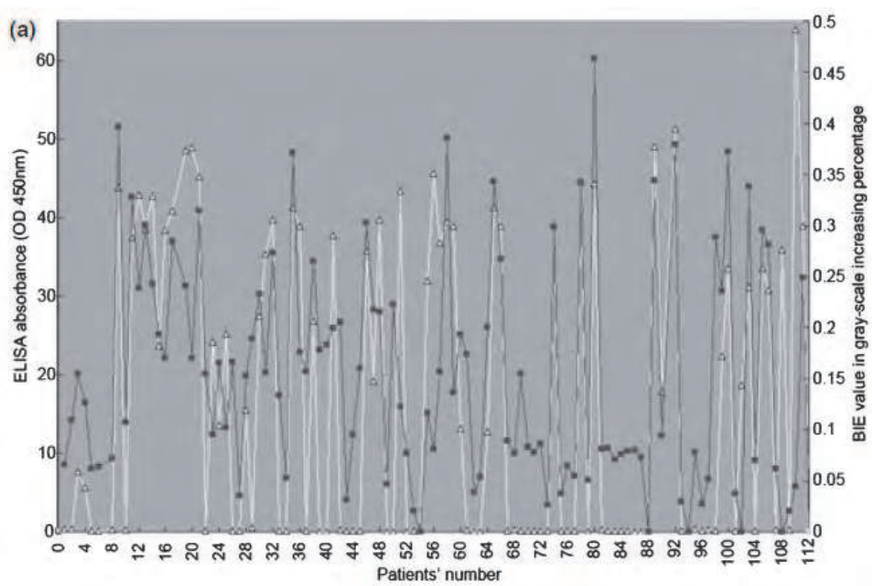


Fig. 11. Comparison of hepatitis B surface antigen detection by the biosensor based on ellipsometry (■) and by enzyme-linked immunosorbent assays (△) (Qi, et al., 2009a).

the Tientsin Blood Disease Hospital. The hepatitis B surface antigen detection results using the biosensor are compared with those of enzyme-linked immunosorbent assays. Regression analysis revealed that the results are in good agreement between the two methods ($r=0.67>r_{0.01}=0.247$).

The biosensor based on ellipsometry allows the multiplexed analysis and detection of five hepatitis B virus markers in clinical samples. The biosensor has a simplified process and short test time, which can detect the five markers from several patients simultaneously in about 1 h. The higher throughput of the biosensor may enable improved setup for detection sensitivity, time, and accuracy in the future.

3.3.2 Quantitative detection of clinical sera from breast cancer patients

Breast cancer incidence rates vary widely across the world, from 19.3 per 100,000 women per year in Eastern Africa to 89.9 per 100,000 women per year in Western Europe (Ferlay, et al., 2010). Carbohydrate antigen 15-3 is particularly valuable for treatment monitoring in patients that have breast cancer that cannot be evaluated using existing radiological procedures. Carbohydrate antigen 15-3 is also used during the postoperative surveillance of asymptomatic women who have undergone surgery for invasive breast cancer.

Using the quantitative calibration curve in section 3.2.2, 60 clinical patients' serum samples were quantitatively analyzed with the biosensor, including 24 women with intraductal carcinoma, 15 with mucinous carcinoma, 5 with in situ lobular carcinoma, 2 with medullary carcinoma, and 14 with breast diseases but no evidence of cancer (Zhang, et al., 2005). Thirty healthy sera were also collected. The median patient age was 48.5 years. These clinical sera samples were examined with both the biosensor based on ellipsometry and electrochemiluminescence immunoassays (Elecsys 2010 system, Roche Diagnostics) via the double-blinded method. The electrochemiluminescence immunoassay is the gold standard of breast cancer marker carbohydrate antigen 15-3 detection. A receiver operating characteristic plot curve (Handley, et al., 1982) was used to determine the result of the biosensor based on ellipsometry, which is shown in Figure 12.

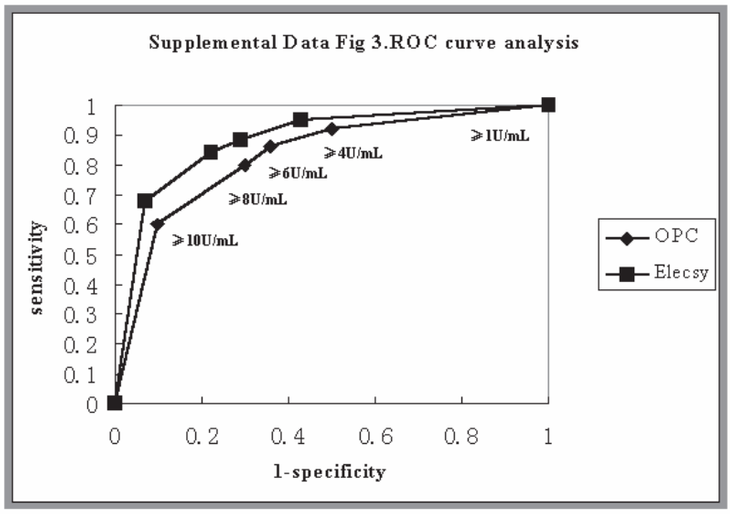


Fig. 12. Receiver operating characteristic curve analysis of the data from the biosensor based on ellipsometry and electrochemiluminescence immunoassays (Zhang, et al., 2005).

The result of this analysis proved that the biosensor results are consistent with those of the electrochemiluminescence immunoassay, reaching the clinical diagnosis standard level.

3.3.3 Clinical detection of sera from severe acute respiratory syndrome coronavirus (SARS-CoV)-infected patients

The outbreak of SARS in late 2002 in southeast China spread rapidly to over 30 countries and resulted in more than 800 deaths (Poutanen, et al., 2003; Feng & Gao, 2007). In 2003, the biosensor based on ellipsometry was used to detect the infectious pathogens.

Before analyzing clinical SARS patients' sera, some antibodies from a phage-display library were identified by the biosensor. SARS-CoV virions were used as a probe by the biosensor to assess the efficiency of the antibodies b1 and h12. The identification of new and effective antibodies is significant for more accurate diagnosis of the illness and the development of a vaccine.

Ten SARS patients and 12 healthy volunteers (controls) were tested with the biosensor. SARS-CoV virions were immobilized on the surface as the probe to detect antibodies in the patients' sera (Figure 13). From the analysis of the results, different patients had different antibody contents, which might help doctors estimate disease progress. The entire detection process only requires approximately 40 min.

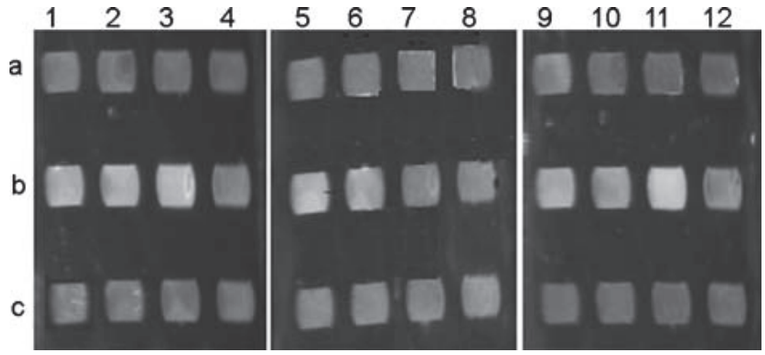


Fig. 13. Analysis of SARS patients' serum samples using the biosensor based on imaging ellipsometry. a1~12 are negative samples; b1~12 are SARS patients; and c1~12 are blank controls (Qi, et al., 2005).

The real-time function of the biosensor was mentioned above in section 3.1.2. The kinetic process of interaction between the antibodies and SARS virus was analyzed with the biosensor. The affinity of antibodies b1 and h12 for SARS virus are 9.5×10^{6} M^{-1} and 1.36×10^{7} M^{-1}, respectively. Real-time detection revealed that antibody h12 has a higher affinity for the virus than antibody b1.

As a label free method, the biosensor based on ellipsometry is a competent mechanism for analyzing clinical serum samples from SARS patients and the affinity between these antibodies and the SARS coronavirus. Compared with surface plasmon resonance (SPR), a fairly widely applied optical detection method for real-time detect interaction of biomolecules (Hall, et al., 2010), the biosensor also allows label-free samples and crude samples to be used directly without previous purification. The biosensor based on ellipsometry has advantages such as: 1) lower cost (e.g., a piece of the biosensor based on ellipsometry silicon wafer is about $1, while a piece of surface plasmon resonance glass slide costs about $70-80); 2) the biosensor can provide 24 real-time curves simultaneously,

allowing high-throughput detection; and 3) multiplex microarray was imaged and offered an image.

3.4 Market potential for scientific research related to the health field

The continual development of the biosensor based on ellipsometry shows both market potential for scientific research related to the health field and an increasing number of applications for basic biology research. The following are two applications of the biosensor on vesicular membrane proteins, demonstrating its value to general biology.

3.4.1 Detection of interaction among vesicular membrane fusion proteins

Membrane-associated proteins provide the minimal fusion machinery necessary for cellular vesicles to fuse to target organelle membranes in eukaryotic cells (Jahn & Scheller, 2006). The qualitative and quantitative identification of membrane-associated proteins interactions is the key to understanding the mechanisms of membrane fusion, which is vital for cell division, cellular structure organization, and biological information processing (Zhang, et al., 2009). To investigate the characteristics of these newly discovered membrane-associated protein pairs such as: Sec22, Ykt6, Sso2 and Sso1, the biosensor based on ellipsometry was used to detect the interactions among soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs, a kind of protein that assembles into coiled-coil tetramers to promote membrane fusion). The interactions among several SNAREs (i.e., Sec22, Ykt6, Sso1, and Sso2) were analyzed by the biosensor based on ellipsometry. The in vitro detection results from the biosensor are consistent with the results of yeast two-hybrid assays at the domain level in vivo (Zhang, et al., 2009; Jin et al., 2011). Further, the kinetic binding process of two SNAREs (Ykt6 and Sso2) was measured using the real-time function of the biosensor. The rapid detection and identification of vesicular protein–protein interactions is essential for understanding vesicle trafficking and for understanding the system-level organization of cellular structure, biological information processing, and molecular mechanisms.

3.4.2 Vesicle adsorption visualization

Recently, a type of total internal reflection imaging ellipsometry was developed for real-time detection of biomolecular interactions (Jin, et al., 2011). This method was used to visualization the of vesicles adsorption process. Non-specific adsorption and desorption on a poly-L-lysine-modified gold surface was analyzed with real-time curves by the biosensor. The biosensor results were consistent with a phase contrast microscopy (NIKON, TI-U, Japan) results. The vesicle adsorption and desorption processes visualized by the biosensor are significant to the study of cell membrane properties. Micron target detection is the future aim of the biosensor based on total internal reflection imaging ellipsometry. Therefore, we expect that the biosensor based on ellipsometry has a yet-unexploited huge market potential for application in biological basic research related to the health field.

4. Summary

In the human health field, the biosensor based on ellipsometry is widely used to monitor or detect biological molecules for applications ranging from common infectious diseases to cancers. Some adaptations of this system for biomedical and clinical applications (e.g., disease marker detection, virus detection, and real-time monitoring) have been developed.

With recent progress on vesicular membrane proteins, the biosensor based on ellipsometry technology also shows significant promise in basic biological research. Furthermore, through miniaturization, it is possible to fabricate the biosensors that are portable, low-cost, high-throughput, and highly sensitive for diseases such as HIV/AIDS. As the biosensor based on ellipsometry becomes simpler and more widely available, we expect to see a proliferation of uses in conjunction with telecommunications equipment. Wide application of the biosensor based on ellipsometry will be explored in monitoring personal health, the food we consume, and our environment in the future.

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A biosensor is a detecting device that combines a transducer with a biologically sensitive and selective component. Biosensors can measure compounds present in the environment, chemical processes, food and human body at low cost if compared with traditional analytical techniques. This book covers a wide range of aspects and issues related to biosensor technology, bringing together researchers from 16 different countries. The book consists of 24 chapters written by 76 authors and divided in three sections: Biosensors Technology and Materials, Biosensors for Health and Biosensors for Environment and Biosecurity.

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